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13. ABSTRACT (Maximum 200 Words)

Non-tumorigenic MCF-10A and tumorigenic MCF-10AT cells were stably transfected with pIRES-polß. DNA polymerase ß overexpression in both cell lines resulted in loss of proliferative potential through apoptosis. Polß overexpression in MCF-10A/AT cells increased the number of apoptotic cells within the population and altered the ratios of pro- and anti-apoptotic protein levels. Polß overexpression in MCF-10A cells resulted in a low incidence of tumors. Polß overexpression in MCF10AT cells resulted in a reduced incidence relative the parental cells, but an increased incidence relative to the vector control cells. The suppression of MCF10AT cell tumorigenicity in the vector transfected controls is likely due to the observed molecular alterations in cell cycle progression proteins induced by puromycin selection. The tumors produced by polß-overexpressing cells displayed an altered histology, relative to the MCF-10AT parental tumors. Molecular analysis of MCF-10A or AT/polß tumors demonstrated that exogenous polß protein expression was diminished or lost in the tumor population. We propose that polß overexpression induces genomic instability in MCF-10A and MCF-10AT cells, resulting primarily in cell death. In rare cells, or within the host environment of the mammary fat pad, cells are selected that have extended proliferative potential, allowing for additional genetic changes and neoplastic progression.

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Introduction

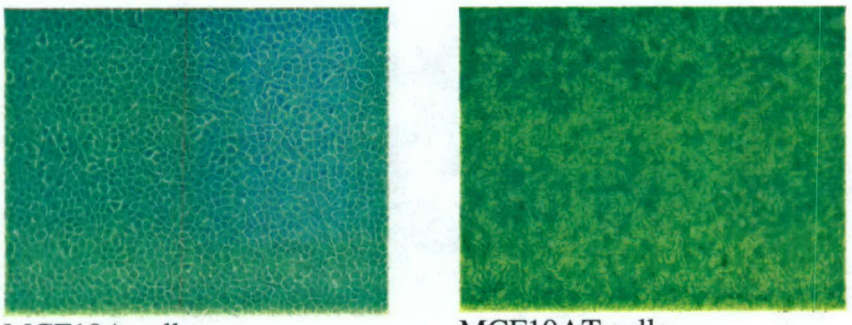
The goal of this proposal was to develop an *ex vivo/in vivo* experimental system to test whether: (i). a mutator phenotype is *sufficient* to accelerate neoplastic transformation of an immortalized breast epithelial cell line, and (ii). a mutator phenotype is *sufficient* to accelerate malignant progression of a neoplastic cell line. In this approach, overexpression of variant mutator forms of DNA polymerase ß (polß) was proposed to act as a surrogate means of generating genetic diversity. Pol ß is the major DNA synthesizing enzyme in base excision repair (BER). DNA lesions resulting from normal cellular metabolism contribute to spontaneous mutations, and are removed by the BER pathway. We hypothesized that intracellular overexpression of wild-type and variant polß enzymes would increase the level of spontaneous mutagenesis, and result in random mutations of oncogene and tumor suppressor loci in epithelial cells. Our long-term goal is to utilize this system to elucidate the role in breast cancer development of various endogenous conditions which may contribute to genetic instability, such as estrogen metabolism and oxidative stress, both of which form DNA adducts repaired by the BER pathway. This avenue of research is vital to understanding oncogenesis in the majority of sporadic human breast cancers, the etiology of which is not associated with familial genetic defects or gross exposures to environmental chemicals.

Body

Task 1: Isolation and characterization of polß overexpressing MCF-10A and -10AT cell lines.

1.A. Establishment of MCF10A and MCF-10AT cell culture.

Ex vivo cell cultures for the MCF-10A and MCF-10AT cell lines were established. We obtained cells from the laboratory of Dr. Danny Welch, co-investigator on this project. We routinely culture the cell lines using published media constituents (Soule *et al.,* 1990): DMEM/F12 media supplemented with 5% horse serum, 10 μg/ml insulin, 10 ng/ml EGF, 0.5 μg/ml hydrocortisone, and 100 ng/ml cholera toxin. Photographs of confluent cultures of each cell line are shown in Figure 1. The *ras*-transfected MCF-10AT cells display the loss of contact inhibition growth patterns expected for a transformed cell line.



MCF10A cells MCF10AT cells

Figure 1. Bright field photographs of parental cell lines used in this study.

1.B. Transfection of MCF10A/AT cells with expression vectors.

B.1. Construction of polß gene overexpression vectors

1.1 *Description of polß expression vectors.* Human cell expression vectors for polß were created by subcloning an NcoI-NotI fragment from our 6X-histidine tagged, rat polß pET bacterial expression vector (Opresko et al, 2002) into two human cell expression vectors (Figure 2).

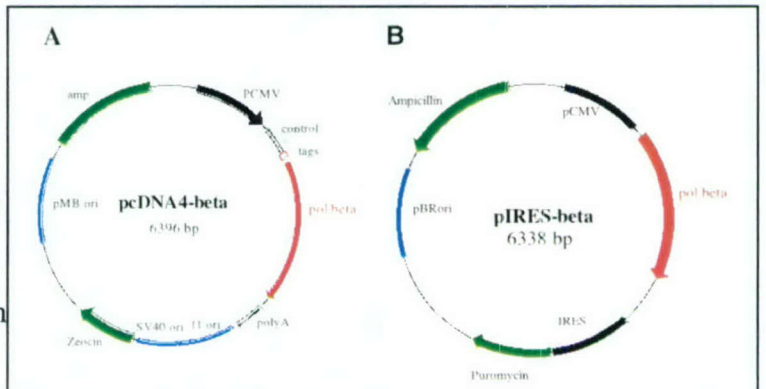


Figure 2. polß expression vectors for MCF-10A/AT cells.

The pIRES puromycin vector (Clontech) vector was chosen for its bicistronic feature which ensures simultaneous expression of the cloned polß gene and the antibiotic resistance gene. The pCDNA4-HisMax (Invitrogen) expression vector was chosen because it encodes two different epitope tags, His and Xpress, for protein detection in human cells. The pIRES-polß expression vector proved to be superior over the pcDNA vector, and has been used in all subsequent experiments included in this Final Report. We proposed to use the expression of variant forms of polß as internal "generators" of genetic diversity. The polß variants Y265W and Y265S have been shown to produce frameshift and base substitution errors at an increased rate, relative to wild-type polß, in an *in vitro* system (Opresko et al, 2002). Three forms of the pIRES/polß vector were created for use in the MCF-10A/AT cell system: wild-type (WT), Y265W and R253M.

B.1.2 Attempts to construct a pol ß gene cassette. Our progress towards creating the epitope-tagged polß expression vectors shown in Figure 2 was slowed by the incompatibility of restriction enzyme sites among the three cloning vectors pET, pIRES and pCDNA4. We attempted a strategy in which new BamHI and BstXI restriction sites could be engineered into the polß insert gene by PCR, thus creating a "pol ß cassette". This cassette then could be moved easily among various vectors, including any we might employ in the future. However, we were unsuccessful in obtaining a full-length polß gene product using this approach (Figure 3). Alterations in all parameters of the PCR, including polymerase, [$MgCl_2$], annealing temperature, and molar ratio of primers and template DNA, did not increase the yield of the desired product. We believe that the forward PCR primer (that includes the BamHI site) also primed internally to the gene to yield a truncated product of ~600 bp in size.

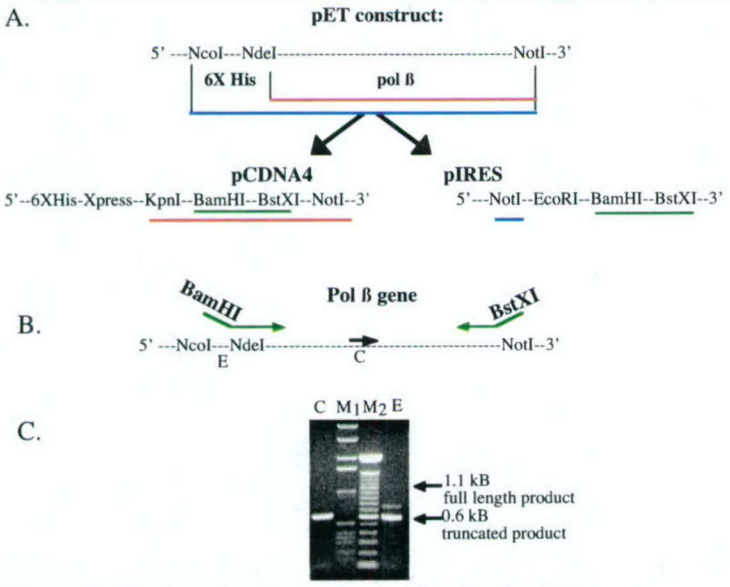


Figure 3. Engineering the polß gene for cloning into mammalian vectors. (A). Schematic of restriction sites in pol ß donor (pET) and recipient (pCDNA4, pIRES) vectors. (B). PCR primers used to introduce new restriction sites (green) into polß gene. (C). PCR products using the BstXI primer and either an internal primer (C) or the experimental BamHI primer (E). M indicates DNA markers.

B.2. Antibiotic cytotoxicity determination.

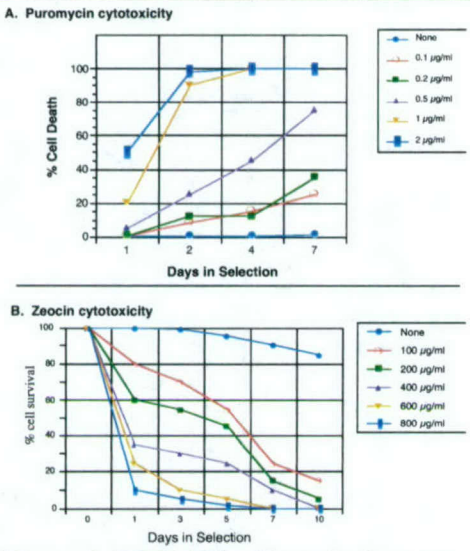


Figure 4. MCF-10A cell survival curves in the presence of selective agents.

Our experimental approach was to isolate stable MCF-10A/AT cell tranfectants, which requires selection for antibiotic resistance. We performed survival curves of MCF-10A cells in the presence of the appropriate antibiotics: Puromycin for the pIRES based vector and Zeocin for the pCDNA based vector. Puromycin is extremely toxic to the MCF-10A cell line (Figure 4A); we observed selection with a minimal dose of 0.5 µg/ml puromycin. Zeocin, a bleomycin derivative is less toxic; we observed a minimal selection dose of ~300 µg/ml Zeocin (Figure 4B). A similar differential was observed for MCF-10AT cells. In our proposal evaluation, one reviewer stated concern for our use of bleomycin as a selective agent, as this antibiotic is known to cause DNA damage and may elevate our mutation frequencies, thus complicating our data analyses. Thus, we used the pIRES vector system, but as will be shown below, the highly toxic nature of puromycin also introduced an unexpected variable into our experiments .

B.3. Optimization of cell transfection

We determined that the passage number of MCF-10A cells significantly influences the efficiency of DNA transfrection (Table 1). In contrast, MCF-10AT cells, a *ras*-transformed derivative of MCF-10A, were highly efficient for DNA transfection, independent of passage number. A second variable which influenced MCF-10A transfection efficiency was the method for introducing DNA into the cells. Electroporation of the DNA was more efficient than lipofection using lipofectin reagent from several commercial sources. Interestingly, the opposite was observed for the transformed MCF-10AT cells, for which lipofection was more efficient than electroporation (Table 1). Other experimental procedures, such as serum starvation or growth factor deprivation, failed to positively impact transfection efficiency for either cell line (Table 1). Finally, we observed a difference in the recovery of puromycin-resistant, stable transfectants when using the parent pIRES vector *versus* the pIRES WTpolß vector. The loss of proliferative potential when polß is overexpressed in MCF10A/AT cells is further addressed in subsequent sections of this report.

Table 1. Optimization of MCF-10A and 10AT DNA transfection

Experimental Variable	Relative Transfection Efficiency	
	MCF-10A	MCF-10AT
1. Passage Number	P96: +	P33: +++
	P98: +	P36: +++
	P101: +++	P38: +++
2. Transfection Method		
Electroporation	+++	+++
Lipofection	++	++++
3. Growth Conditions		
Complete	+++	+++
Serum depleted	+	++
Growth factor and serum depleted	-	+
4. Vector		
pIRES	+++	++++
pIRES WTpolß	++	++

1.C. Measure levels of polß protein expression by Western analyses.

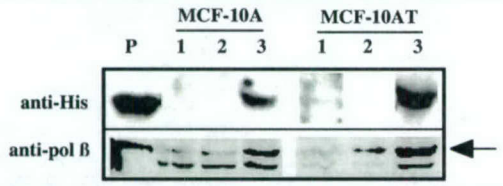


Figure 5. WT Polß expression in MCF-10A and 10AT cells transfected with pIRES vectors. Exogenous polß is detected by the anti-His antibody (top panel). Total cellular polß protein is detected by the anti-polß antibody (bottom panel, arrow). P, pure protein; lane 1, parental cells; lane 2, pIRES transfected cells; lane 3, pIRESWTpolß-transfected cells.

Western analyses were performed using anti-polß antibody (NeoMarkers) and cell extracts from the pCDNA-ß clones, pCDNA4 (vector) clones and untransfected MCF10A cells. Endogenous polß was readily detected in all samples. However, no protein was detected using the same samples and probing with an anti-His antibody (Qiagen) to detect exogenous polß expression from the pCDNA-ß vector (not shown). The optimization experiments described above resulted in robust expression of exogenous WTpolß from the pIRES vector in both MCF-10A and MCF-10AT cells (Figure 5, top panel, lane 3). Total polß protein is increased 1.8-fold in MCF-10A/ pIRES WTpolß cells and 2.8- fold in MCF-10AT/ pIRES WTpolß cells, relative to the corresponding pIRES controls (Figure 5, bottom panel, lane 2 *versus* lane 3). We were unsuccessful in isolating stable transfectants overexpressing the Y265W/polß variant.

1.D. Characterization of polß-overexpressing cell lines.

We consistently observed that MCF-10A cells overexpressing WTpolß failed to proliferate as cell populations after two passages post-transfection. The MCF-10AT/WTpolß population also lost viability, albeit at a later passage. This failure was not observed in the pIRES vector transfected controls, indicating that the loss of proliferative potential is a result of polß overexpression. Viability was maintained in early and mid passages of WTpolß-overexpressing cell populations (Figure 6). Loss of viability was accompanied by a dramatic change in cell morphology characterized by rounded, floating cells and giant adherent cells (Figure 7, compare with Figure 1). The observed lethality of polß overexpression in MCF10A/AT breast epithelial cells may be cell-type specific. Polß overexpression in

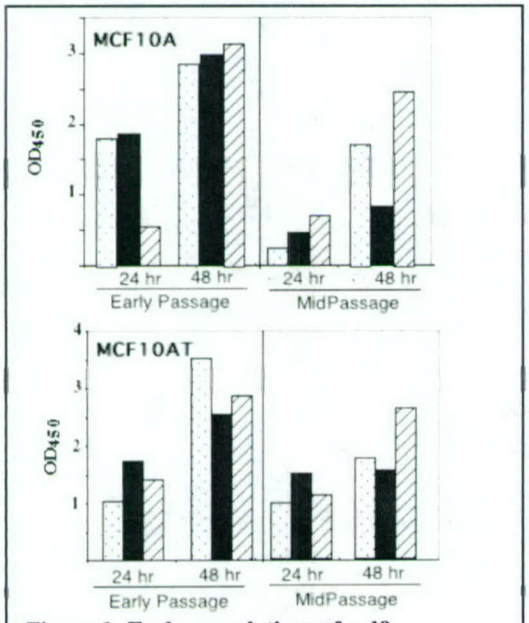


Figure 6. Early populations of polß overexpressing cells are viable. Cellular dehydrogenase assay as a function of time. Stippled bars, parent cells; solid bars, vector only transfectants; striped bars, pol beta transfectants.

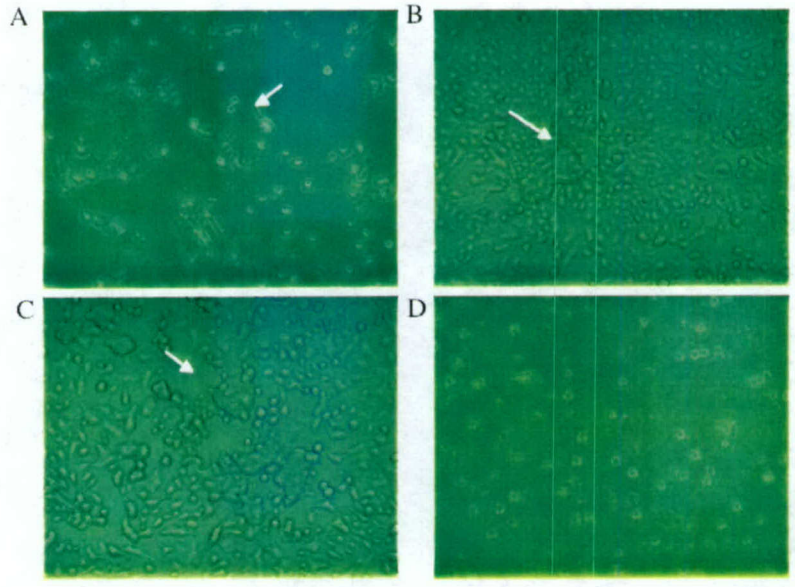


Figure 7. Altered cell morphology induced by overexpression of polß. Panels A-C, MCF10A/pIRESpolß expressing cells, 2-3 passages post selection; Panel D, MCF10AT/pIRESpolß expressing cells, 3 passages post selection. Arrows point to rounded, floating cells and adherent giant cells.

CHO cells (Canitro et al, 1998) and h-TERT immortalized human fibroblasts (Yamada and Farber, 2002) results in genetic instability; no loss of proliferative potential was noted in these papers. In a recent publication, however, Fotiadou et al (2004) describe teleomere dysfunction produced by overexpression of pol ß in a non-transformed murine mammary cell line. These investigators also describe the production of giant cells and loss of proliferative potential.

Task 2: Quantitation of HSV-tk mutation rates in control and polß-overexpressing MCF cell lines.

The oriP-tk shuttle vector pJY102 was constructed previously for use in epithelial cell lines. This vector contains the oriP and EBNA-1 sequences from Epstein-Barr virus for episomal replication in epithelial cells; the hygromycin resistance gene for selection of vector-containing human cells; and the thymidine kinase gene from Herpes simplex virus type 1 for mutational analyses. In our proposal, we intended to use the pJY102 vector to quantitate the HSV-tk mutation rate in polß-overexpressing cell populations 30 and 60 passages post-transfection. The observed loss of proliferative potential in MCF10A and MCF10AT cells expressing WTpolß precluded our ability to perform these mutational analyses.

Task 3: Determine *in vitro* transformation properties of polß overexpressing cell populations

We proposed to determine the extent of growth factor independence and the efficiency of anchorage-independent growth in polß expressing cell populations after ~30 and ~60 passages. The lack of cell proliferation after 3-4 passages required us to undertake an alternative strategy to characterize the cell populations.

3. A. Molecular changes in transfected cell populations

We analyzed the degree to which puromycin selection and polß overexpression altered MCF-10A and MCF-10AT cells by Western analyses of a variety of molecular markers. A portion of these analyses are displayed in Figure 8 and summarized in Table 2. For all cases, we analyzed adherent cell populations three passages post-transfection.

A.1. *Effects of puromycin (parental cells versus vector-only transformants).* MCF-10A cells are extremely sensitive to the toxic effects of puromycin, and this cytotoxicity is reflected in the significant stabilization of p53 protein in the pIRES vector transfected MCF-10A cell population, relative to the untransfected parental cells. A much smaller effect of puromycin selection on p53 status in MCF-10AT cells was noted (two-fold), consistent with the transformed phenotype of this cell line. The increased p53 levels were not accompanied by an increased level of Ser15 phosphorylation, but were accompanied by a five-fold increase in the level of p21 (Waf1), a transcriptional target of p53. The c-myc transcription factor levels were slightly elevated in the vector-transfected cells. Puromycin also increased the levels of activated ras in MCF-10AT cells (1.4-fold). Together, these data suggest that puromycin selection has altered cell cycle checkpoints and mitogenic signalling in the target cells.

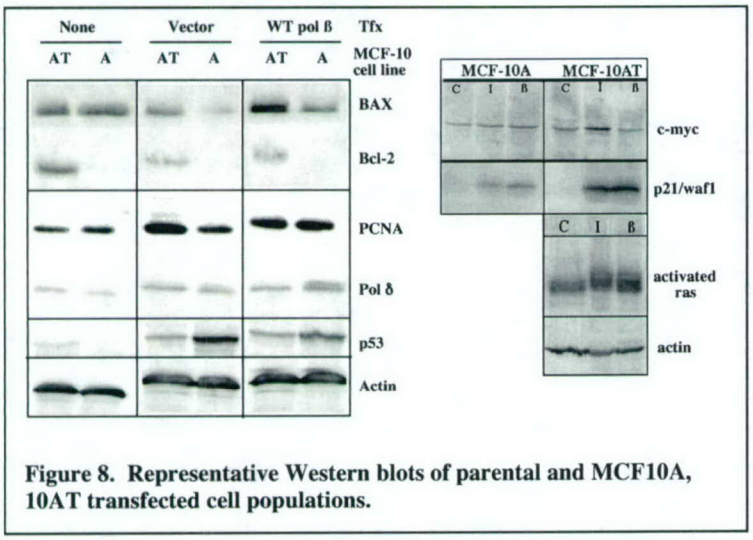


Figure 8. Representative Western blots of parental and MCF10A, 10AT transfected cell populations.

A.2. *Effects of polß overexpression on DNA repair proteins.*

Table 2. Phenotypic effect of WTpolß expression on MCF-10A and MCF-10AT cells: Western blot analyses

Pathway/Protein	Relative change of protein in polß-overexpressing cells [a]	
	MCF-10A	MCF-10AT
DNA Repair		
Mismatch repair / MSH2	4 x ↑	none
Double strand break repair / Rad50	none	none
Base excision repair /		
APE-1	none	none
PCNA	2 x ↑	2x ↓
pol delta	1.8 x ↑ *	none
Apoptosis		
p53	none	none
Bax	4 x ↑	2 x ↑
Bcl-2	not dectectable	none
Chromatin Structure		
RCC-1	3 x ↑	none

[a] Fold change relative to pIRES vector-transfected cells. Increases or decreases of less than 50% are indicted as "none".
* Indicates presence of new molecular weight protein species.

Polß is the rate-limiting enzyme in the base excision repair pathway. The levels of three other proteins in the base excision repair pathway were also examined: APE-1, PCNA, and DNA polymerase delta (pol δ). The most notable change in this pathway was the increased levels of PCNA and pol δ in MCF-10/WTpolß expressing cells, relative to vector controls, and the concomitant appearance of a new form of pol δ (Figure 8). The new molecular weight species may represent a phosphorylated form of the protein. These changes were not observed in MCF-10AT cell populations. We examined proteins of the mismatch repair pathway (MSH2), which removes DNA polymerase errors, and the double strand break repair pathway (RAD50), which repairs DNA breaks causing chromosomal rearrangements. A notable increase in MSH2 protein level (4.2-fold) was observed in polß overexpressing MCF-10A cells, relative to the vector control, but no such change was observed in MCF-10AT cells (Table 2). This result may be due to cellular compensation for an increased number of DNA mismatches caused by polß. No changes in RAD50 levels were observed for either cell line. RCC-1 is a chromatin-associated protein implicated in nuclear formation, nuclear transport, transcription and DNA replication.

A.3. *Increased apoptosis in polß-overexpressing cells.* Western analyses revealed alterations in pro and anti-apoptotic proteins in polß overexpressing cell populations. We can detect no anti-apoptotic Bcl-2 protein in any of the MCF10A cell lines, while the pro-apoptotic Bax protein is increased four-fold in MCF-10A cells expressing WTpolß, relative to vector controls (Fig.8). Unlike MCF-10A cells, MCF-10AT cells constitutively express detectable levels of Bcl-2 protein. The levels of Bax protein are also increased in the MCF-10AT/polß overexpressing cells. The net effect is the alteration of the Bax:Bcl-2 ratio in polß overexpressing cells to favor the pro-apoptotic Bax.

We performed functional studies using the AnnexinV marker to confirm that these molecular changes result in the induction of apoptosis in polß overexpressing cell populations. We observed an elevated proportion of early apoptotic cells in polß-overexpressing MCF10A and AT cells, relative to the vector controls, and the proportion increased with passage number (Figure 9A). The MCF-10A cell line is more sensitive to the effects of polß overexpression than the MCF-10AT cell line, as demonstrated by the high number of late apoptotic/dead cells in this population at early passage (Figure 9B).

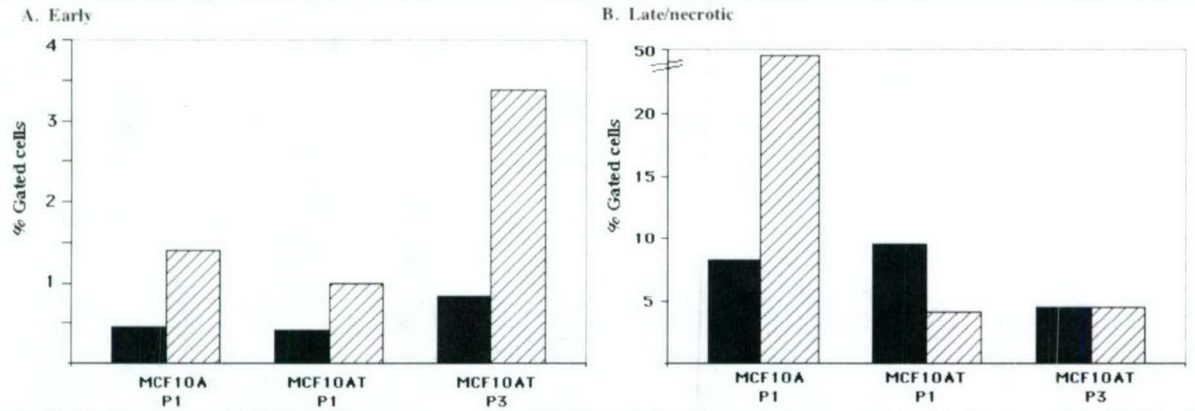


Figure 9. Induction of apoptosis by polß overexpression. The indicated cell populations were stained with AnnexinV-FITC and propidium iodide (PI), and analyzed by flow cytometry. A. Percentage of gated cells in early apoptosis (Annexin-positive, PI-negative); B. Percentage of gated cells in late apoptosis/necrosis (Annexin positive, PI positive). Solid bars, vector transfected; hatched bars, polß transfected cells.

3. B. Effects of polß-overexpression on MNU cytotoxicity. We tested whether increased levels of polß affect the efficiency of BER by measuring killing of MCF-10A/AT populations by N-methyl-N-nitrosourea (MNU). Repair of cytotoxic O^6-methylguanine lesions can also be mediated by mismatch repair. We previously reported increased levels of MSH2 in polß over-expressing cells. We did not observe a significant change in the MNU cytotoxicity curves for either cell population (figure 10). We conclude that polß overexpression neither enhances nor interferes with BER in MCF10A/AT cells.

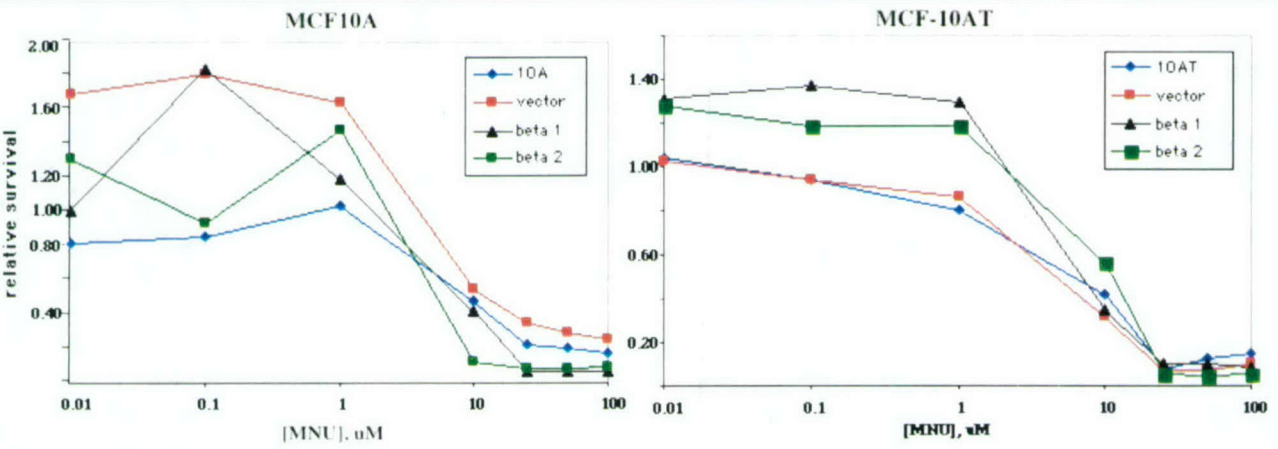


Figure 10. MNU cytotoxicity in control and polß-overexpressing cell populations. Cells were treated for 1 hr at the indicated concentrations of MNU and assayed for viability 24hrs later. Survival is relative to untreated cells in each group.

Task 4: Quantitate mammary tumor incidence and latency in control and polß-overexpressing MCF-10A/AT cell lines.

4.A. Tumor incidence and characterization

Early passage, puromycin-selected cell populations were used for the tumorigenicity experiments. Cells were injected into the mammary fat pad of nude mice, and the formation of tumors monitored over several months. Parental (unselected) MCF-10A cells did not form tumors, while we observed an overall tumor incidence of 38% for the *ras*-transformed MCF10AT parental cell line (Table 3). MCF-10AT cells are reported to be tumorigenic; intriguingly, our cell line appeared to lose its tumorigenic potential with time in culture, as the absolute incidence varied from 80% to 8% from 2001 to 2003. The MCF-10AT tumors arose beginning 50 days after mammary fat pad injection, and ranged in size from 6-17 mm in diameter at time of removal or sacrifice. In three independent experiments, we observed one or no tumors produced by MCF10AT cells transfected with the pIRES vector only control (Table 3). This suppression is likely due to the observed molecular changes induced by puromycin, such as increased p53 and p21 levels (Figure 8).

Tumors were observed after injection of both MCF10A/polß and MCF-10AT/polß overexpressing cells. We observed three small (5-6 mm) tumors arising from MCF10A/polß, corresponding to an incidence of 12% ; we observed on overall 31% incidence of tumors after injection of MCF10AT/ polß cells (Figure 12 and Table 3). The MCF10AT/polß tumors arose with a latency period similar to that of the parental cells, but were smaller than those observed in the parental controls.

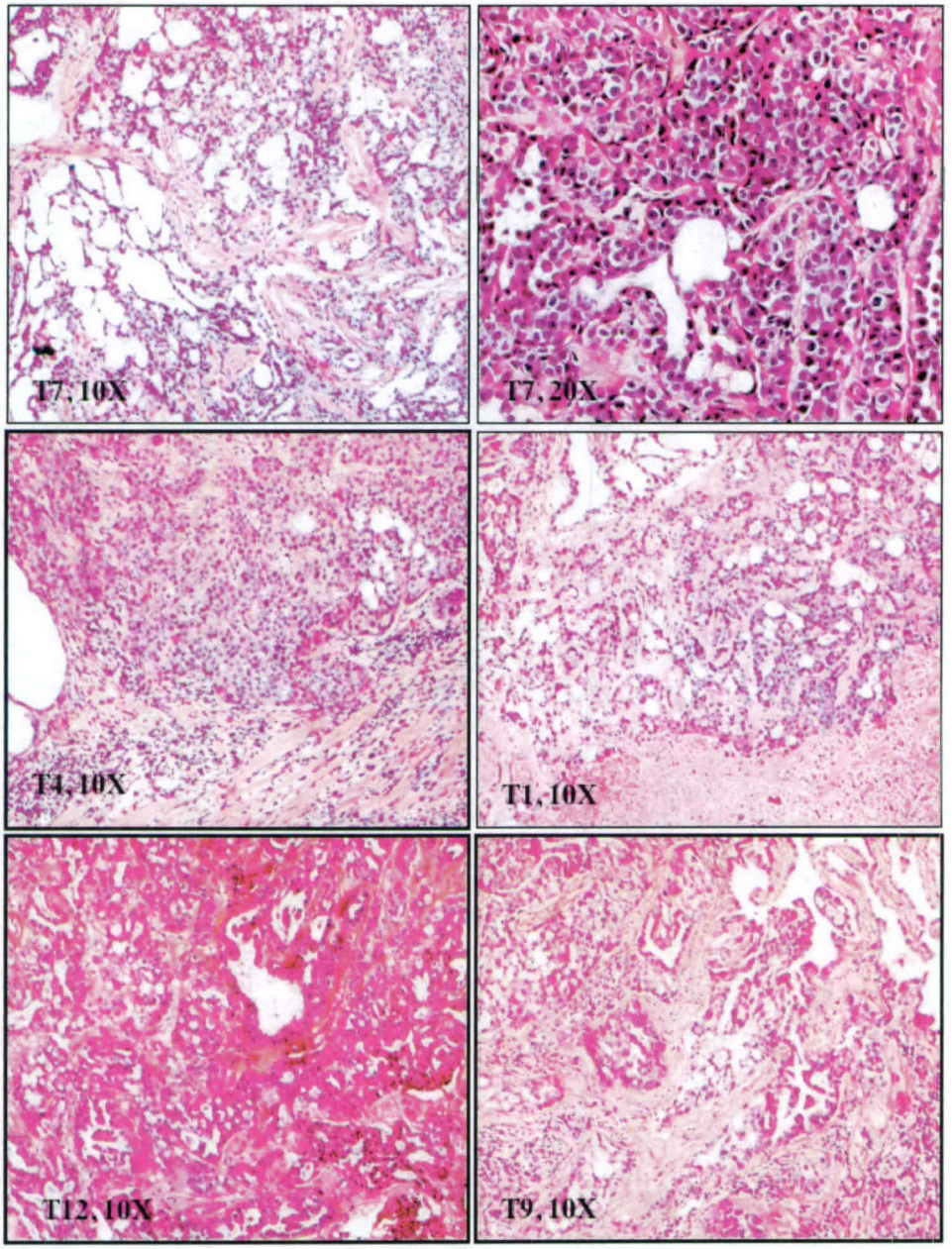
Table 3. Mammary tumor incidence of MCF-10A and MCF-10AT parental and polß-transfected cells .

Cell line / vector	Injections [a]		Tumorigenicity[b]	
	Group	N mice	Incidence	Appearance
MCF-10A	01-257a 02-1100 total	4 12 16	0 0	n.a.
MCF-10A / pIRES	01-257c	5	0	n.a.
MCF-10A / pIRESWTpolß	02-207 02-159c 02-159d total	8 8 8 24	0 0 3 3 (12%)	 floating mass
MCF-10AT	01-257b 02-159a 03-100a total	5 4 12 21	4 3 1 8 (38%)	 solid
MCF-10AT / pIRES	01-257d 02-415 03-100b total	5 8 8 21	0 1 0 1 (4.8%)	 solid
MCF-10AT / pIRESWTpolß	01-257e 02-159b 03-100c total	5 8 16 29	3 1 0 4 (14%)	fluid filled

[a] Mammary fat pad injection of 1 x 10^6 cells in nude mice.

[b] Primary tumor at least 2mm x 2mm in size after 175-236 days

Figure 11. Hemotoxylin and eosin stained histologic sections of independent tumors arising after m.f.p. injections of MCF-10AT cells.



Histologically, the MCF-10AT parental tumors were solid and displayed infiltration of the stromal layer (Figure 11). In contrast, The tumors arising in both the MCF-10A/polß and MCF-10AT/WTpolß groups were fluid filled and less solid than those arising from the MCF-10AT parental cells (Figure 13). The tumors were encapsulated, with well-defined boundaries, and the MCF-10 cells were arranged in defined islands within the stroma. In some tumors, we observed extensive lymphocytic infiltration. These images demonstrate that the tumors formed after puromycin selection and overexpression of polß differ from those formed by the parental cells.

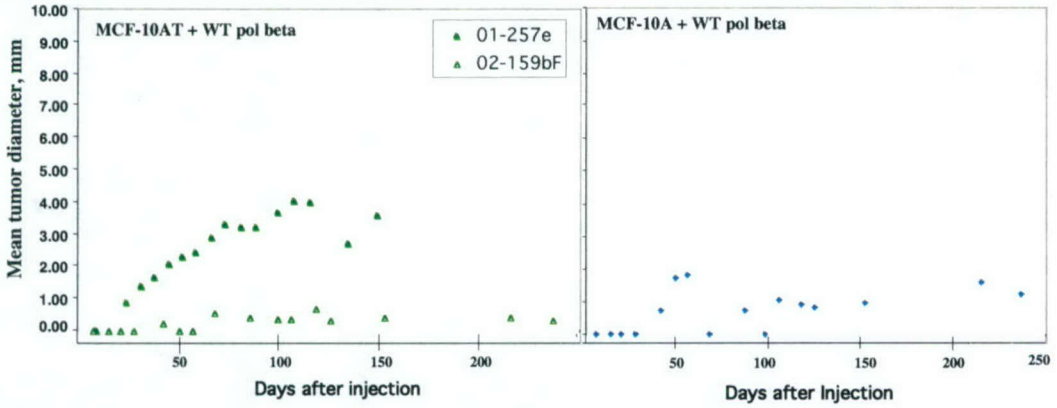


Figure 12. Tumorigenicity of MCF-10AT/polß or MCF-10A/polß cell populations. Mean tumor diameter of all animals in group is recorded. Groups contained 4-8 animals. Tumors were removed when size measured 10mm.

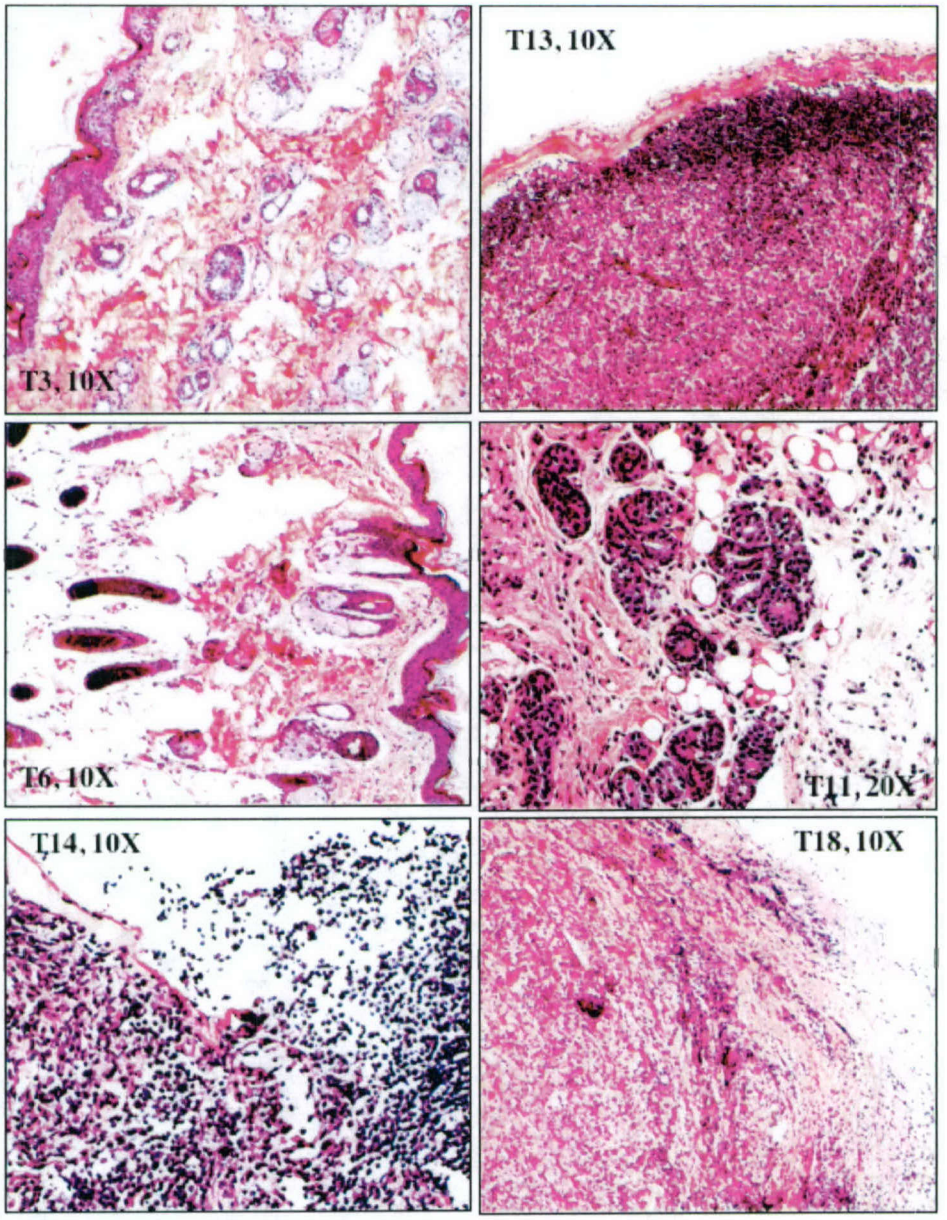


Figure 13. Mammary tumors formed after injection of MCF10AT/polß cells (left panels) and MCF-10A/pol ß cells (right panels).

4.B. Molecular characterization of mammary tumors

Lysates were prepared from mammary tumors of each group, and analyzed for expression of the exogenous polß using an anti-His antibody (see Figure 3). Endogenous polß was detected in most samples; however, the his-tagged polß protein is either absent or below detection in all lysates (Figure 14). This result raises the possibility the tumors in Figure 13 resulted from outgrowth/selection of MCF10A/AT cells that lost polß expression after injection. In addition to frozen tissue specimens, paraffin-embedded sections were used for immunohistochemal analyses of polß expression in the tumors. Consistent with the Western analyses, we did not observe consistent, differential polß staining in tumors from MCF-10AT parental versus MCF10AT/polß cells (Figure 15). In some MCF-10AT/polß tumor sections, we did observe intense polß staining in isolated islands of cells (Fig. 15, panel E). We also observed increased staining of endothelial cells and infiltrating cells (panels C and F). We conclude that a low percentage of MCF10A/AT-polß cells retain ectopic polß expression in the tumors, but the majority of cells no longer overexpress pol ß.

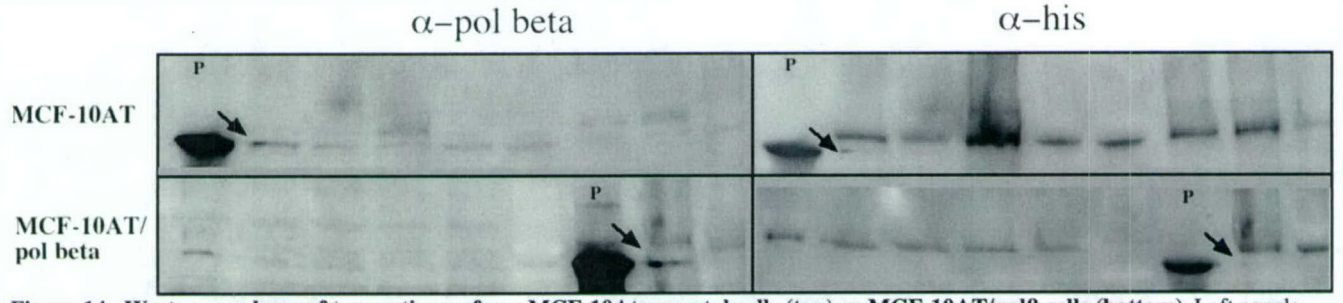


Figure 14. Western analyses of tumor tissues from MCF-10At parental cells (top) or MCF-10AT/polß cells (bottom). Left panels, anti-polß; right panels, anti-his. P, purified his-tagged polß protein. Arrows point to position of expected polß protein. The exogenous, his-tagged polß protein is undetectable in tumors from parental MCF10AT cells or from polß expressing cells.

Task 5: Observe metastatic potential of control and polß-overexpressing MCF-10A/AT cell lines.

We observed lung metastases in 2 of 3 tumor-bearing mice derived from MCF-10AT/WTpolß expressing cells. The tumors were too small to collect for further experimental examination. Thus, we have insufficient data to conclude whether these metastases were spontaneous or were induced by polß-overexpression.

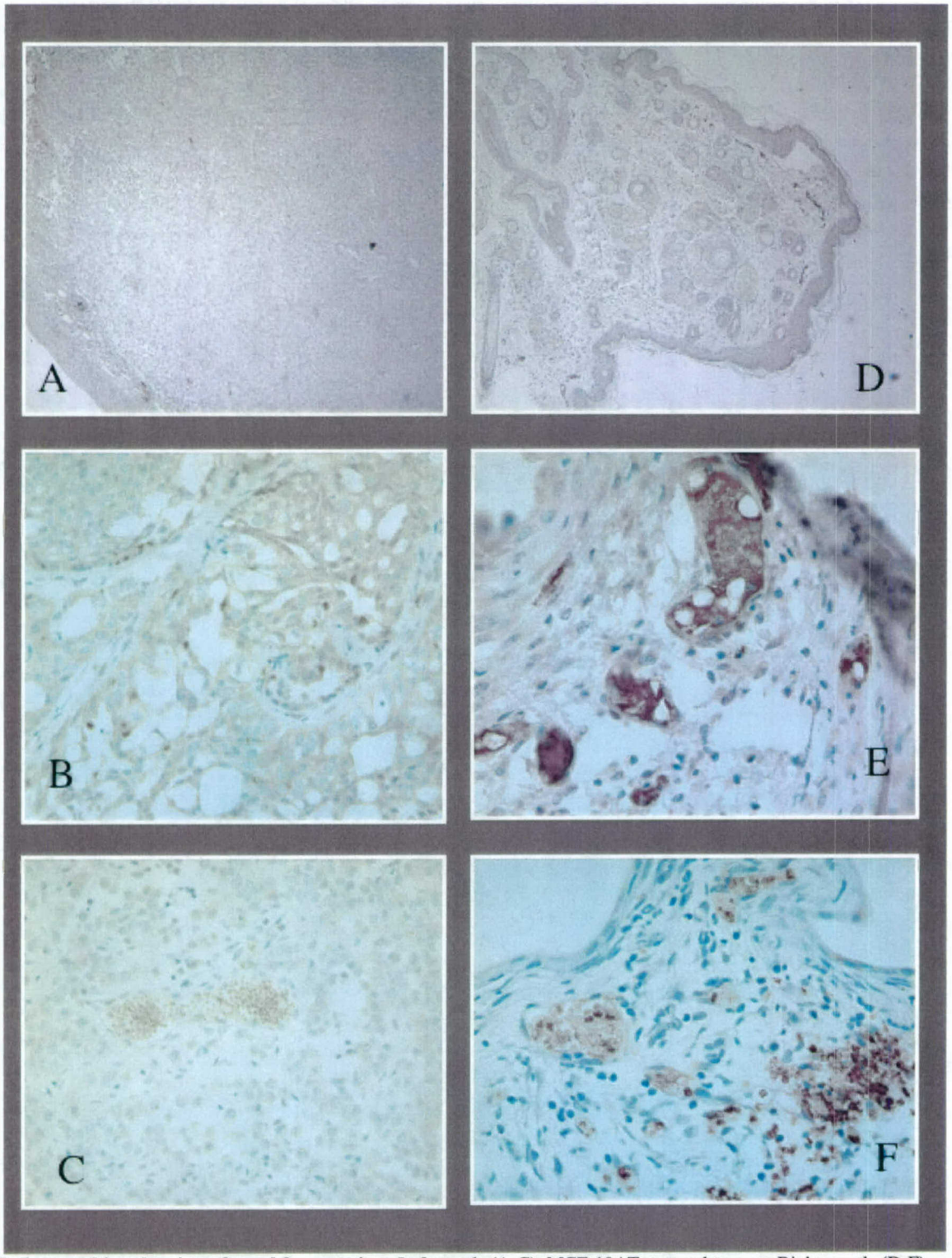


Figure 15. Immunohistochemistry for pol ß expression. Left panels (A-C), MCF-10AT parental tumors. Right panels (D-F), MCF10AT/polß tumors. Tumors were incubated with rabbit anti-polß primary antibody and biotinylated anti-goat secondary antibody; reactions were developed with DAB (hematoxylin counterstain).

Key Research Accomplishments

- Establishment of MCF-10A and MCF-10AT cell culture systems.
- Isolation and characterization of WTpolß-overexpressing MCF-10A and MCF-10AT cell populations.
- Demonstrated increased apoptosis in WTpolß-overexpressing cell populations with limited proliferative potential, concomitant with altered levels of several proteins involved in the control of cell proliferation and death.
- Determined the effects of polß overexpression on MNU-induced cytotoxicity of MCF10A/AT cells.
- Quantitated tumorigenicity of MCF10A/AT parental cells, vector-transfected cells and polß overexpressing cells.

Reportable Outcomes

Abstracts:

Vineeta Khare, Danny Welch, and Kristin Eckert (2002) Overexpression of DNA polymerase beta in MCF-10AT cells reduces tumorigenicity in nude mice, Poster presentation, Department of Defense "Era of Hope" meeting, Orlando, FL

Manuscript in preparation:

Khare, V., Hile, S.E., Yan, G., Welch, D.R. and K. A. Eckert, Overexpression of DNA polymerase beta induces apoptosis and alters tumorigenicity of MCF-10AT human breast epithelial cells.

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Personnel List

Salaries of the following personnel were supported by this award:

1. Dr. Kristin Eckert, Associate Professor, Principal Investigator
2. Dr. Danny R. Welch, Associate Professor, Co-Investigator
3. Dr. Vineeta Khare, Post-doctoral Fellow
4. Ms. Guang Yan, Senior Research Technician
5. Ms. Kathy Lipman, Research Support Technician I

Conclusions

We have observed that overexpression of DNA polymerase ß in diploid, human breast epithelial cell lines, MCF-10A and MCF-10AT, results in loss of proliferative potential, through the induction of apoptosis. The response may, in part, result from the antibiotic (puromycin) used for selection of the transfected cells. The *ras*-transformed MCF-10AT cell line appears to be somewhat more tolerant of polß overexpression. Polß overexpression in MCF-10A/AT cells increases the number of apoptotic cells within the population and may alter the control of apoptosis, as evidenced by aberrant ratios of pro- and anti-apoptotic protein levels. This apoptotic response may be due to the dysregulation of telomere maintenance that has been reported to result from polß overexpression in murine mammary cells (Fotiadou et al, 2004).

Polß overexpression in MCF-10A cells resulted in a low incidence of tumors (12%), whereas no tumors were observed from the parental cell line. The tumors arising from MCF10AT cells overexpressing polß displayed a reduced incidence (14%) relative the parental cells (38%), but an increased incidence relative to the vector control cells (4.8%). The suppression of MCF10AT cell tumorigenicity in the vector transfected controls is likely due to the observed molecular alterations in cell cycle progression proteins induced by puromycin selection. The tumors produced by polß-overexpressing cells displayed an altered histology, relative to the MCF-10AT parental tumors. These tumors were cyst-like rather than solid, displaying lympocyte infiltration in some cases. Two animals bearing MCF-10AT/polß tumors progressed to form lung metastases. Molecular analyses of tumor lysates arising from the MCF-10AT/WTpolß cells demonstrated that exogenous polß protein expression was diminished or lost in the tumor populations. Immunohistochemical analyses indicate that polß overexpression may be retained in isolated islands of cells within the tumor. These observations suggest that biologic selection occurred within the animal for a MCF-10AT/WTpolß cell variants that have lost polß overexpression. We propose that polß overexpression induces genomic instability in MCF-10A and MCF-10AT cells, and that these genomic alterations (presumably chromosome aberrations) result primarily in cell death. In rare cells, or within the host environment of the mammary fat pad, variants are selected that have the potential to proliferate for an extended period of time, allowing for additional genetic changes and neoplastic progression.

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